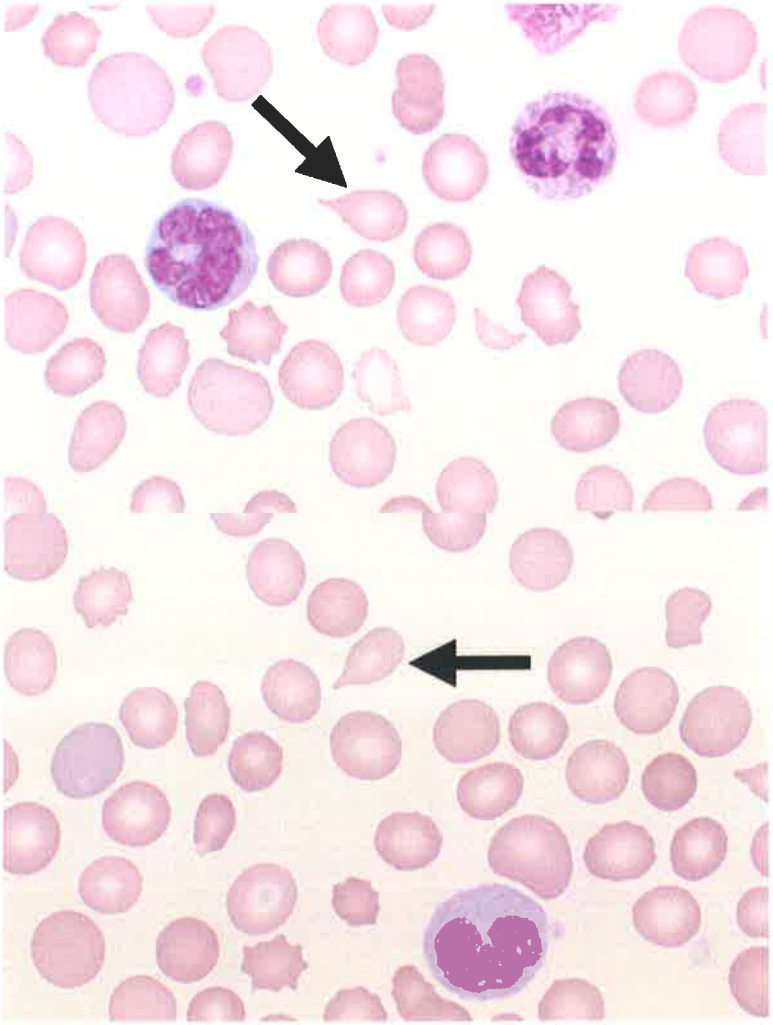
Cell Identification

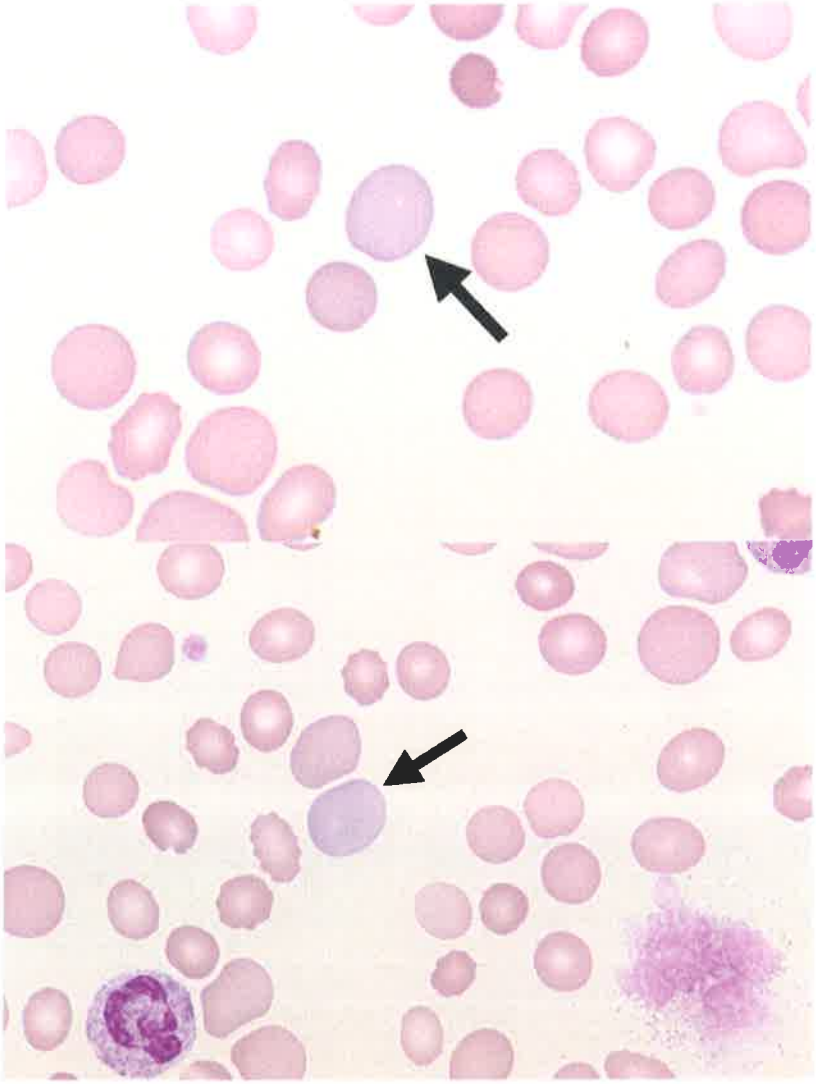
VPBS-02



Identification	Participants No.	%	Evaluation
Teardrop cell (dacrocyte)	1159	99.5	Educational
Target cell (codocyte)	3	0.3	Educational
Pappenheimer bodies (iron or Wright stain)	2	0.2	Educational
Neutrophil, segmented or band	1	0.1	Educational

The arrowed cells are tear drop cells (dacrocytes), as correctly identified by 99.5% of the participants. Red blood cells appearing in the shape of a teardrop or a pear with a single, short or long, often blunted or rounded end are called teardrop cells (dacrocytes). These are commonly seen in patients with bone marrow fibrosis, but may also be seen in pernicious anemia, anemia of renal disease, hemolytic anemias (as in our patient), and other forms of severe anemia. These cells are often associated with an abnormal spleen or bone marrow. Bone marrow infiltration with hematologic and non-hematologic malignancies may also be accompanied by dacrocytosis. Teardrop cells may be seen as an artifact of slide preparation; such dacrocytes are usually easily recognized due to the fact that their "tails" all point in the same direction. In our slide, the "tails" point in different directions.

VPBS-03



Identification	Participants No.	Participants %	Evaluation
Polychromatophilic (non-nucleated) red blood cell	1119	96.0	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	37	3.2	Educational
Hypochromasia	3	0.3	Educational
Ovalocyte (elliptocyte)	2	0.2	Educational
Spherocyte	2	0.2	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational

The arrowed cells are polychromatophilic (non-nucleated) red blood cells, as correctly identified by 96.0% of the participants. A polychromatophilic red blood cell is a non-nucleated, round, or ovoid red blood cell that represents the final stage of red blood cell maturation after exiting the bone marrow. . It is larger than a mature erythrocyte and usually lacks central pallor. It primarily contains hemoglobin with a small amount of RNA, and thereby stains homogeneously pink-gray or pale purple with a Romanowsky or Wright-Giemsa stain.

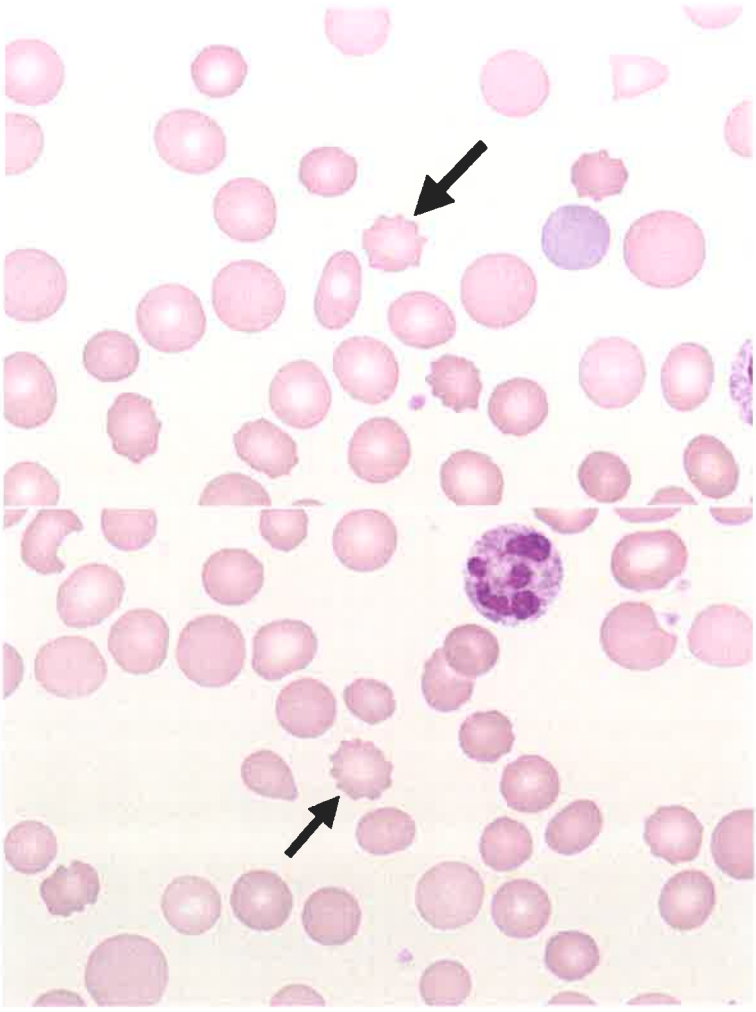
VPBS-03, (cont.)

VPBS-03 Discussion, Cont'd:

These cells can be stained as reticulocytes and enumerated by using supravital stains, such as new methylene blue. With supravital staining, reticulocytes reveal deep blue granular and/or filamentous structures. This reticulin network is called the "substantia reticulofilamentosa." The amount of precipitated RNA and intensity of polychromasia varies inversely with the age of the reticulocyte. Automated technologies for assessing reticulocytes improve the accuracy and precision of determining reticulocyte numbers. Polychromatic macrocytes are frequently increased in patients with hemolysis.

3.2% of participants identified these arrowed cells as macrocytes, oval/round. Macrocytes are abnormally large red blood cells (diameter > 8.5 µm). They are best detected by comparing to other red blood cells in a smear in the context of the MCV. They may be oval or round. The hemoglobin concentration is normal; cells lack significant polychromasia (ie, if polychromasia is readily identified, the term polychromatophilic red blood cell is preferred for proficiency testing purposes). As the arrowed cells have a distinct gray, pale purple color as compared to the surrounding red blood cells, the choice of macrocyte is incorrect, and polychromatophilic red blood cell is preferred.

VPBS-04

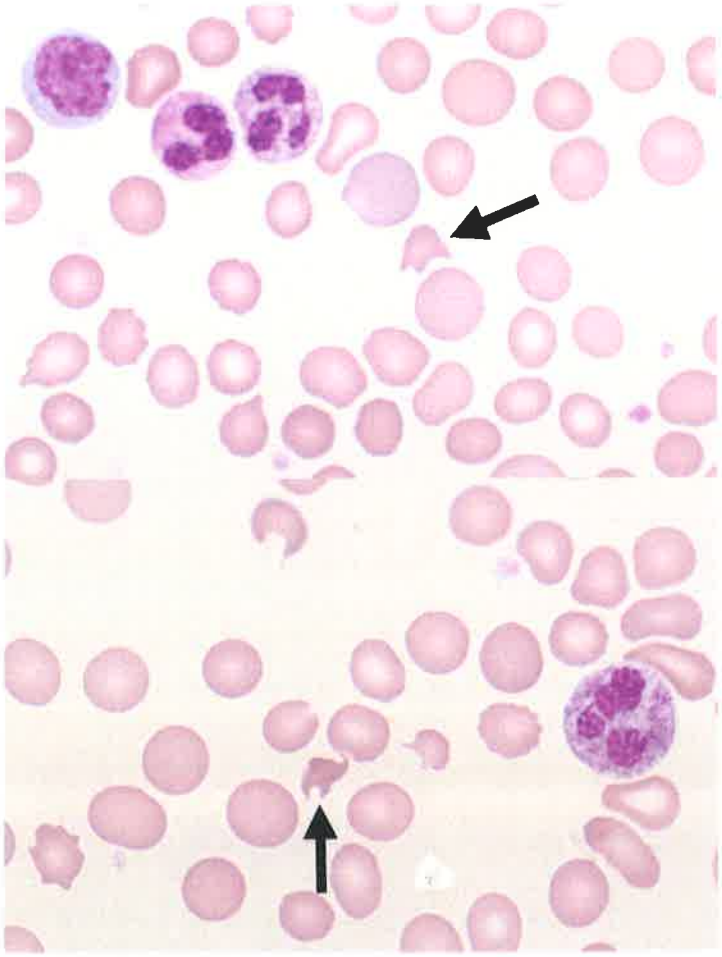


Identification	Participants No.	Participants %	Evaluation
Echinocyte (burr cell, crenated cell)	1149	98.6	Educational
Acanthocyte (spur cell)	15	1.3	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational

The arrowed cells are echinocytes (burr cell, created cell), as correctly identified by 98.6% of the participants. Echinocytes are red blood cells with 10 - 30 uniform, short, blunt projections distributed evenly that impart a serrated appearance to the red blood cell surface. The red blood cells retain central pallor and are the same size or slightly smaller than normal red blood cells. Their appearance is often the result of an improperly prepared smear (slow drying, thick smears, aged blood, and pH alteration of glass slide). Echinocytes that are not artifacts may be indicative of disease, such as uremia or pyruvate kinase deficiency, post splenectomy, in hepatitis of the newborn, and phosphoglycerate kinase deficiency. Under such circumstances, they should be visible in wet preparations.

1.3% of participants identified the arrowed cells as acanthocytes (spur cells). Acanthocytes are densely stained, spheroidal red blood cells that lack central pallor and have multiple (usually three to 20), irregularly distributed, thorn-like spicules of variable size, often with drumstick ends. As the arrowed cells retain central pallor and show relatively uniform, evenly spaced projections, echinocyte is correct and acanthocyte is incorrect.

VPBS-05



Identification	Participants No.	%	Evaluation
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1130	97.0	Educational
Acanthocyte (spur cell)	29	2.5	Educational
Bite cell (degmacyte)	5	0.4	Educational
Monocyte	1	0.1	Educational

The arrowed cells are fragmented red blood cells (schistocytes), as correctly identified by 97.0% of the participants. Fragmented red blood cells are red blood cells that have undergone rips and tears when draped over fibrin strands in the microcirculation or have suffered buffeting against unyielding structures in the macrocirculation (such as artificial heart valves). Fragments resulting from such trauma reseal by fusion of opposing ends and persist in the circulation, presumably for a short time. Fragmented red blood cells include helmet cells, keratocytes (horn cells), triangulocytes, and a more inclusive term, schistocytes.

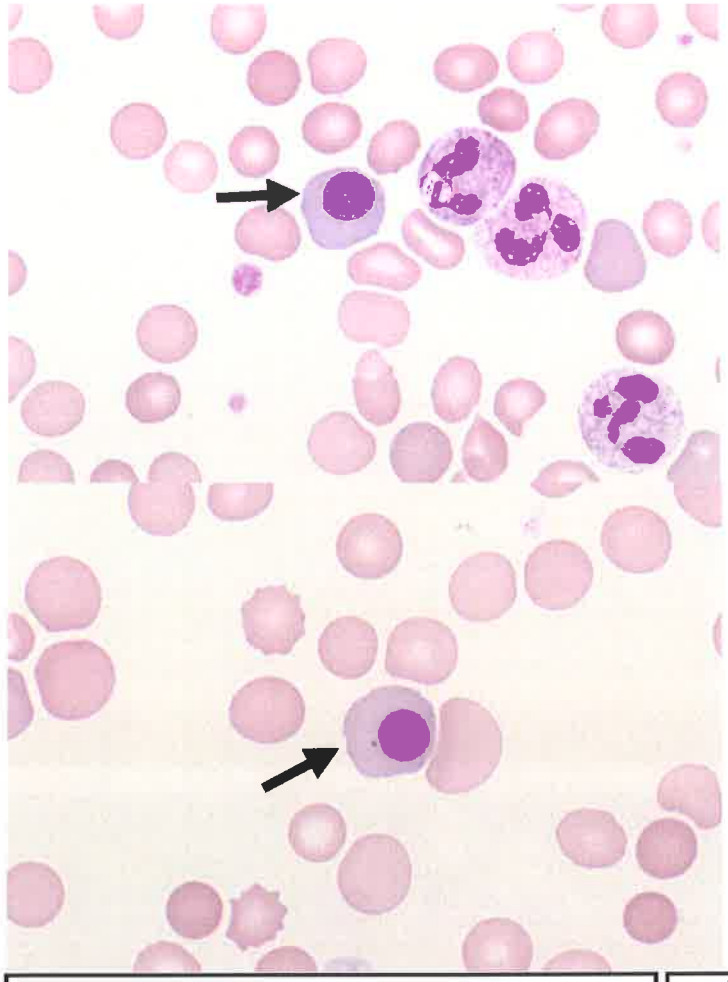
A zone of central pallor is rarely present in fragmented cells. Occasional spherocytes are almost invariably present in association with fragmented cells. These spherocytes are the product of the rounded-up red blood cell fragments.

VPBS-05 Discussion, Cont'd:

Fragmented cells are seen in severe burns, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), and other microangiopathic hemolytic anemias, in patients with prosthetic cardiac valves or severe valvular stenosis, malignant hypertension, or other mechanical trauma to the cell (eg, march hemoglobinuria, marathon running). When present in large numbers, they may cause the MCV to fall into the microcytic range or interfere with platelet enumeration.

2.5% of participants identified the arrowed cells as acanthocytes (spur cells). Acanthocytes are densely stained, spheroidal red blood cells that lack central pallor and have multiple (usually three to 20), irregularly distributed, thorn-like spicules of variable size, often with drumstick ends. The arrowed cells are not spheroidal, but instead appear as pieces of a red blood cell, ie, fragments and are therefore consistent with fragmented red blood cells and not acanthocytes.

VPBS-06



Identification	Participants No.	%	Evaluation
Nucleated red blood cell, normal or abnormal morphology	1160	99.6	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	2	0.2	Educational
Eosinophil, any stage	1	0.1	Educational
Hemoglobin C crystal	1	0.1	Educational
Monocyte	1	0.1	Educational

The arrowed cells are nucleated red blood cells, as correctly identified by 99.6% of the participants. The term nucleated red blood cell (nRBC) is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the circulating nucleated red blood cell is at the orthochromic stage of differentiation. Both megaloblastic and dysplastic changes can be seen in these circulating red blood cells, reflecting simultaneous erythroid maturation abnormalities present in the bone marrow. Caution should be used in classifying a circulating nucleated red blood cell as dysplastic on the basis of abnormal nuclear shape (lobated or fragmented), as these changes may occur during their egress from the marrow space and may not be present in the maturing erythroid precursors present in the marrow. For the purposes of proficiency testing, it is adequate to identify a cell as a nucleated red blood cell when it is present in the peripheral blood, be it normal or abnormal (ie, exhibits megaloblastic or dysplastic changes). nRBC may be seen in the peripheral blood of patients with on-going hemolysis.

Clinical Presentation:

The peripheral blood smear is from a 66-year-old woman presenting with gram negative sepsis, now oozing from intravenous sites. Laboratory data include: WBC = 52.7 x 10E9/L; RBC = 2.69 x 10E12/L; HGB = 8.8 g/dL; HCT = 28.0%; MCV = 104 fL; PLT = 80 x 10E9/L; and RDW = 25%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Disseminated intravascular coagulation

Disseminated intravascular coagulation (DIC) is a form of microangiopathic hemolytic anemia that is due to systemic activation of the coagulation cascade and fibrinolytic pathway. The activation of the coagulation cascade may potentially result in thrombotic obstruction of small to medium caliber vessels resulting in organ dysfunction. As this occurs, platelets and coagulation proteins are consumed, which may cause hemorrhagic complications. Therefore, a spectrum of presentations is possible with approximately 5 - 12% of patients presenting with extreme bleeding. This aforementioned etiology differentiates DIC from other forms of microangiopathic hemolytic anemia such as thrombotic thrombocytopenic purpura, which is usually due to autoantibodies against ADAMTS-13, hemolytic uremic syndrome, which is frequently due to a Shiga toxin, and atypical hemolytic uremic syndrome, which is due to uncontrolled activation of the complement system.

Question 1. Disseminated intravascular coagulation is due to?

A. A germline mutation resulting in uncontrolled activation of the complement system
B. A Shiga-like toxin and therefore is often seen in association with diarrhea, fever, and vomiting
C. Abnormal systemic activation of the coagulation cascade and fibrinolytic cascade resulting in thrombotic and hemorrhagic complications
D. An autoantibody against ADAMTS-13 resulting in large weight multimers of von Willebrand factor in the blood

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DIC is always seen in association with an underlying condition. Therefore, the treatment of DIC is management of the underlying cause. In addition, replacement of platelets, plasma, and coagulation factor concentrates may be needed. In certain cases, heparin may be utilized.

Common associations include severe infections (historically gram negative sepsis though gram positive sepsis is potentially just as likely), malignancy (including hematologic malignancies such as acute promyelocytic leukemia and solid tumors such as metastatic pancreatic mucinous adenocarcinoma), significant trauma (such as severe burns or orthopedic trauma), or obstetric complications (such as amniotic fluid emboli).

Upwards of 35% of causes of severe sepsis are complicated by DIC, which significantly increases mortality. Factors involved in the development of DIC associated with sepsis include microbial membrane components, which may activate the coagulation cascade, or exotoxins that may cause a strong immune response and release of cytokines. In these cases, the diagnosis of DIC may be challenging as anemia and thrombocytopenia are not uncommon in the setting of acute infection.

Specifically, in patients with classic DIC, expected laboratory values include prolonged aPTT and PT with increased LDH and d-dimer, and suppressed haptoglobin and fibrinogen. Like other microangiopathic hemolytic anemias, anemia, thrombocytopenia, and schistocytes on manual smear inspection are typically expected. aPTT and PT are prolonged due to consumption of clotting proteins during clot formation, which also explains the suppressed fibrinogen and thrombocytopenia. LDH is increased due to red cell shearing also resulting in anemia with schistocytes. Haptoglobin is suppressed as hemoglobin is released from sheared red blood cells. D-dimer is increased due to clot lysis and is felt to be the most sensitive, but certainly not specific, marker of DIC. As certain clotting proteins, such as Factor VIII and fibrinogen, LDH, haptoglobin, and d-dimer are all acute phase reactants, laboratory interpretation can be challenging and therefore, clinical correlation is needed.

Question 2. Which laboratory value is frequently suppressed in disseminated intravascular coagulation?

A. aPTT
B. D-dimer
C. Haptoglobin
D. LDH

Question 3. Which clinical/laboratory scenario would be most typical for disseminated intravascular coagulation?

A. A patient is diagnosed with metastatic mucinous adenocarcinoma and now has oozing at their IV site; anemia and thrombocytopenia are noted on CBC with schistocytes seen on blood smear
B. A patient sustains severe burns over 70% of their body; laboratory work-up reveals isolated anemia without thrombocytopenia; coagulation testing reveals a suppressed aPTT
C. An obstetric patient suffers an amniotic fluid emboli and now has shortness of breath; CBC data are normal except for a mild microcytic anemia which had been attributed to iron deficiency
D. An otherwise healthy female presents acutely with altered mental status and vague abdominal pain; anemia and thrombocytopenia are noted on CBC with schistocytes seen on blood smear; coagulation testing

Natasha Savage, MD
Hematology and Clinical Microscopy Committee

REFERENCES:

1. Levi M, Scully M. How I treat disseminated intravascular coagulation. *Blood.* 2018;131(8):845-854.
2. Levi M, van der Poll T. Coagulation and sepsis. *Thromb Res.* 2017;149:38-44.

Answers to Questions:

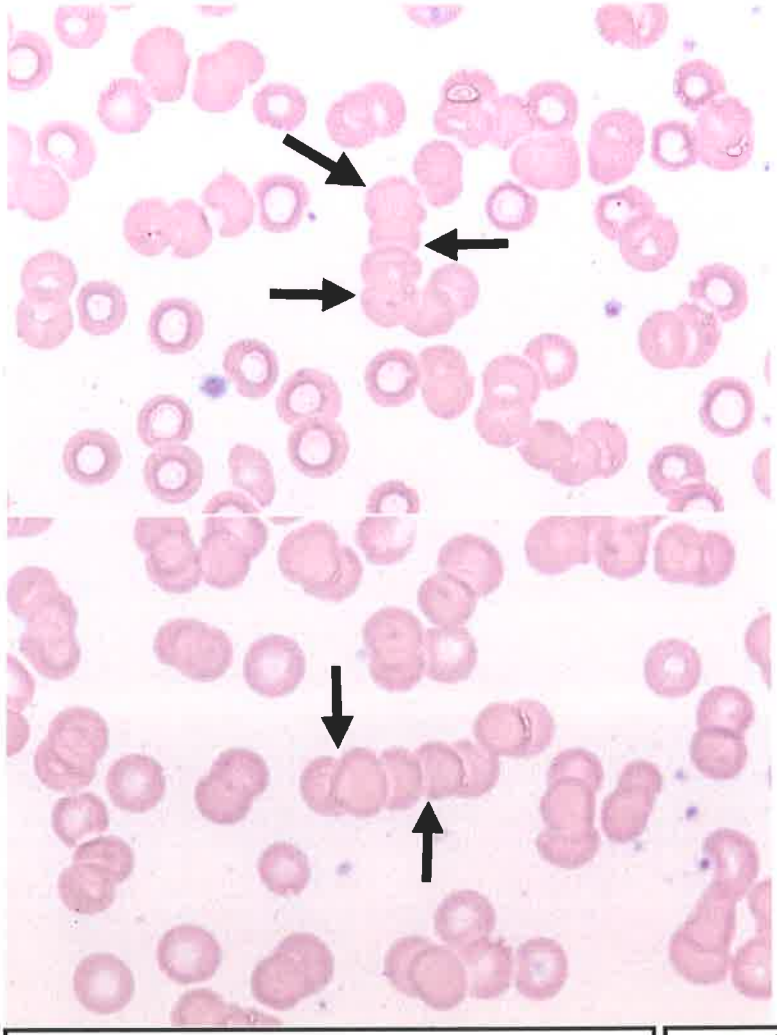
Question 1: C. Abnormal systemic activation of the coagulation cascade and fibrinolytic cascade resulting in thrombotic and hemorrhagic complications.
Hemolytic uremic syndrome is frequently due to a Shiga-like toxin associated with diarrhea, fever, and vomiting. Thrombotic thrombocytopenic purpura is usually due to an autoantibody against ADAMTS-13 resulting in large weight multimers of von Willebrand factor in the blood. Atypical hemolytic uremic syndrome is frequently due to a germline mutation resulting in uncontrolled activation of the complement system.

Question 2: C. Haptoglobin
All other values are usually elevated. aPTT is increased due to consumption of clotting cascade proteins. D-dimer is elevated due to clot lysis. LDH is elevated due to red cell shearing resulting in schistocytes.

Question 3: A. A patient is diagnosed with metastatic mucinous adenocarcinoma and now has oozing at their IV site; anemia and thrombocytopenia are noted on CBC with schistocytes seen on blood smear
Scenario B does not have features of DIC given lack of thrombocytopenia and lack of increased aPTT. Scenario C does not have features of DIC given lack of thrombocytopenia. Scenario D has features of thrombotic thrombocytopenic purpura. The acute presentation without obvious underlying clinical cause (ie, sepsis, trauma, malignancy, etc.) and normal coagulation testing help exclude DIC.

Cell Identification

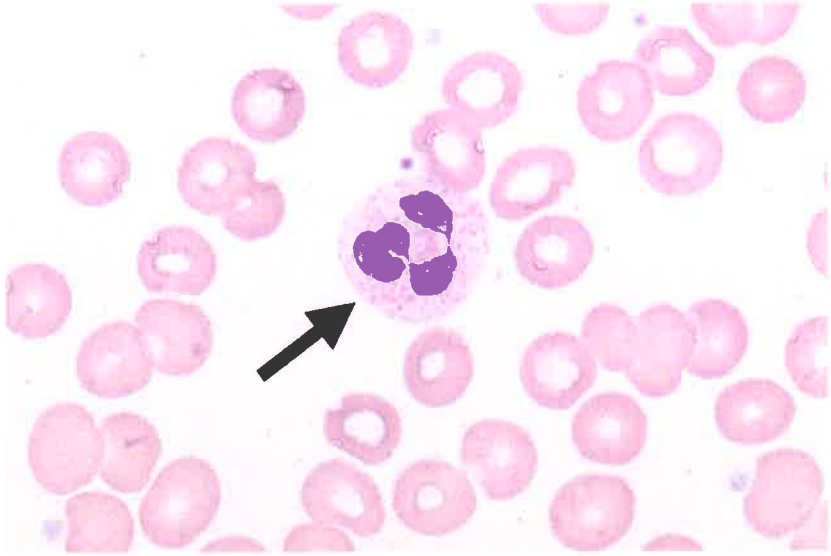
VPBS-08



Identification	Participants No.	Participants %	Evaluation
Rouleaux	1163	99.9	Educational
Red blood cell agglutinates	1	0.1	Educational

The arrowed objects are red blood cells demonstrating rouleaux formation, as correctly identified by 99.9% of participants. This term describes the appearance of four or more red blood cells organized in a linear arrangement that simulates a stack of coins. The length of this arrangement (18 µm or more) will exceed its width (7 to 8 µm), which is the diameter of a single red cell. The central pallor of the red blood cells is obscured due to overlapping of the cells' cytoplasm, however, it is apparent in singly placed red blood cells. True rouleaux formation is present when seen in the thin area of a blood film and is due to increased amounts of plasma proteins, primarily fibrinogen, and globulins. In this case Rouleaux formation is associated with monoclonal gammopathy due to Waldenstrom macroglobulinemia. Rouleaux formation is a normal finding when rouleaux noted in only the thick area of a blood film.

VPBS-09

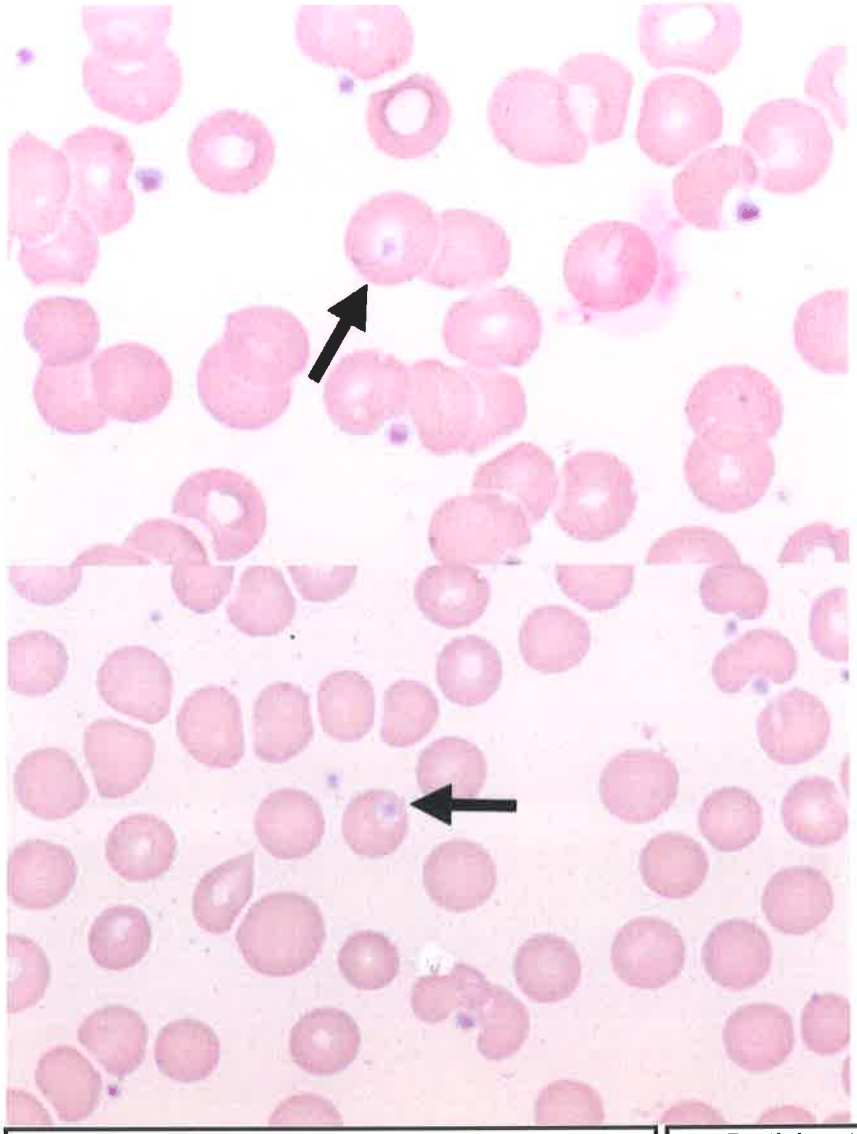


Identification	Participants No.	%	Evaluation
Neutrophil, segmented or band	1126	96.7	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	25	2.1	Educational
Neutrophil with hypersegmented nucleus	6	0.5	Educational
Eosinophil, any stage	3	0.3	Educational
Acanthocyte (spur cell)	1	0.1	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed object is a segmented neutrophil, as correctly identified by 96.7% of participants. Segmented neutrophils are typically the predominant leukocyte in the peripheral blood. They are usually about 10 to 15 µm in diameter, round to oval, with segmented to lobated nuclei (typically, three to five lobes), with pale pink cytoplasm with specific granules. The chromatin is highly condensed, and the lobes are connected by solid, dark, thread-like filaments with no internal chromatin. For the purposes of proficiency testing, band and segmented neutrophils are identified together.

2.1% of participants incorrectly identified the arrowed cell as a toxic neutrophil. Toxic changes in neutrophils include toxic granulation. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes, and the arrowed neutrophils contain fine eosinophilic granules. Neither toxic vacuolization nor Döhle bodies are present.

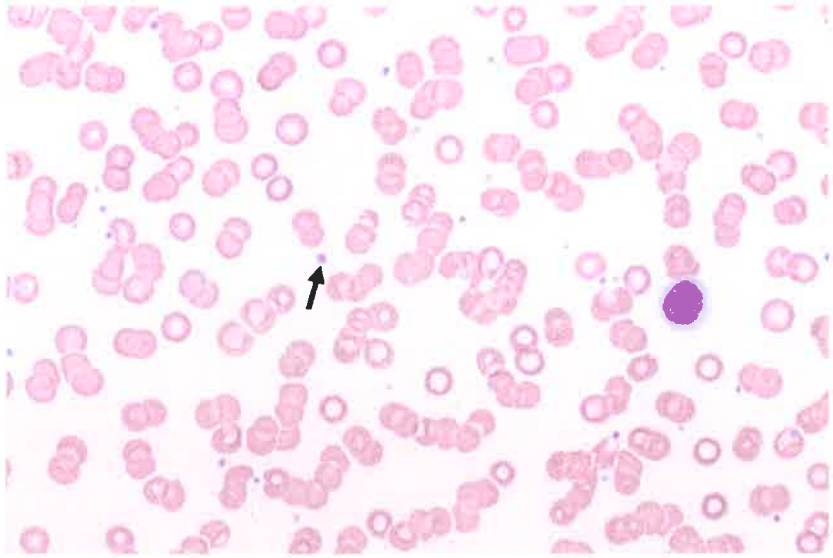
VPBS-10



Identification	Participants No.	%	Evaluation
Erythrocyte with overlying platelet	1144	98.3	Educational
Platelet, normal	6	0.5	Educational
Howell-Jolly body	5	0.4	Educational
Erythrocyte, normal	4	0.3	Educational
Hypochromasia	2	0.2	Educational
Pappenheimer bodies (iron or Wright stain)	1	0.1	Educational
Platelet, hypogranular	1	0.1	Educational
Target cell (codocyte)	1	0.1	Educational

The arrowed objects are erythrocytes with overlying platelet, as correctly identified by 98.3% of participants. In preparing a peripheral blood smear, platelets may adhere to or overlap red blood cells, suggesting a red blood cell inclusion or parasite. A correct interpretation depends on carefully examining the morphology of the platelet and comparing the size, staining characteristics, and granularity with known platelets in the same field as well as determining if the platelet is in the same plane of focus as the red blood cell. Often times, the platelet is surrounded by a thin clear zone or halo, which is not a feature of most genuine red blood cell inclusions.

VPBS-11

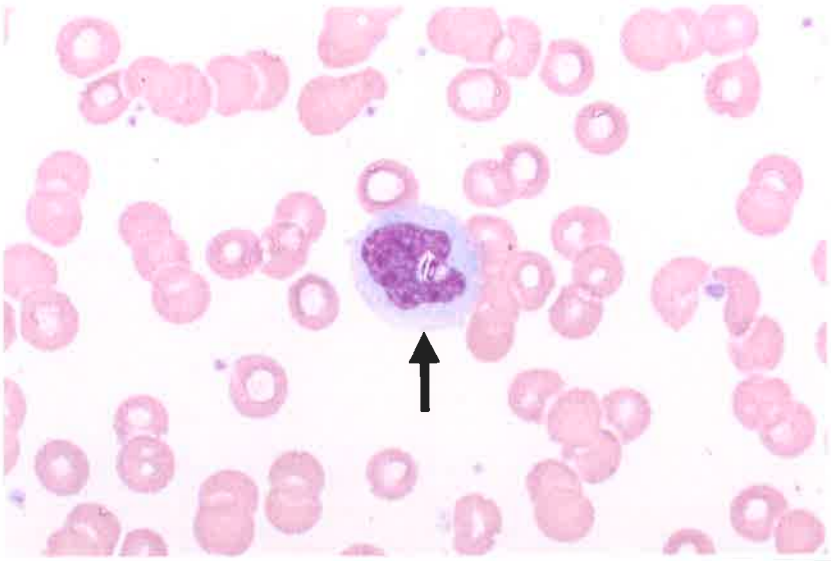


Identification	Participants No.	%	Evaluation
Platelet, normal	1141	98.0	Educational
Platelet, giant (macrothrombocyte)	14	1.2	Educational
Platelet, hypogranular	4	0.3	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	2	0.2	Educational
Erythrocyte, normal	1	0.1	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.1	Educational
Platelet satellitism	1	0.1	Educational

The arrowed object is a platelet, as correctly identified by 98.0% of participants. Platelets (also known as thrombocytes) are blue-gray fragments of megakaryocytic cytoplasm, which play an essential role in primary hemostasis. In the peripheral blood of healthy individuals, platelets are small and fairly uniform in size, most measuring 1.5 to 3 µm in diameter, with a few smaller forms (less than 1.5 µm) and a few larger forms (4 to 7 µm). Fine, purple-red, alpha granules are dispersed throughout the cytoplasm or are sometimes aggregated at the center.

1.2% of participants incorrectly identified the arrowed cell as a giant platelet. Giant platelets are larger than 7 µm, usually measuring 10 to 20 µm in diameter. For proficiency testing purposes, the term giant platelet is used when the platelet is larger than the size of the average red blood cell in the field. The arrowed platelet is much smaller that red blood cells in the field.

VPBS-12



Identification	Participants No.	%	Evaluation
Monocyte	1096	94.2	Educational
Monocyte, immature (promonocyte, monoblast)	22	1.9	Educational
Neutrophil, metamyelocyte	19	1.6	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	10	0.9	Educational
Malignant lymphoid cell (other than blast)	6	0.5	Educational
Neutrophil, giant band or giant metamyelocyte	3	0.3	Educational
Neutrophil, segmented or band	3	0.3	Educational
Immature or abnormal cell, would refer for identification	2	0.2	Educational
Neutrophil, myelocyte	1	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.1	Educational

The arrowed object is a monocyte, as correctly identified by 94.2% of participants. Monocytes are slightly larger than neutrophils, ranging from 12 to 20 µm in diameter. The majority of monocytes are round with smooth edges, but some may have pseudopod-like cytoplasmic extensions. The cytoplasm is abundant, with a gray or gray-blue ground-glass appearance, and may contain vacuoles or fine, evenly distributed azurophilic granules. The N:C ratio ranges from 4:1 to 2:1. The nucleus is usually indented, often resembling a three-pointed hat, but it can also be folded or band-like. The chromatin is condensed; however, it is usually less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent; however, occasional monocytes may contain a small, inconspicuous nucleolus.

1.9% of participants incorrectly identified the arrowed cell as an immature monocyte (promonocytes or monoblasts). The malignant monoblast is a large cell, usually 15 to 25 µm in diameter with the N:C ratio ranging from 7:1 to 3:1. The monoblast nucleus is round or oval and has finely dispersed chromatin and distinct nucleoli. The cytoplasm is blue to gray-blue and may contain small, scattered azurophilic granules. Promonocytes have nuclear and cytoplasmic characteristics that are between those of monoblasts and mature monocytes. They are generally larger than mature monocytes, but they have similar-appearing gray-blue cytoplasm that often contains uniformly distributed, fine azurophilic granules. The nuclei show varying degrees of lobulation and are characterized by delicate folding or

VPBS-12, (cont.)

VPBS-12 Discussion, Cont'd:

creasing of the nuclear membrane, in contrast to the rounder nuclear profile of monoblasts or mature monocyte. Nucleoli are present but may not be as distinct as in monoblasts.

1.6% of participants incorrectly identified the arrowed cell is a neutrophil, metamyelocyte. Metamyelocytes are approximately 10 to 18 µm in diameter with round to oval with a N:C ratio of 1.5:1 to 1:1. The nuclear chromatin is condensed and the nucleus is indented to less than half of the maximal nuclear diameter (ie, the indentation is smaller than half of the distance to the farthest nuclear margin). The cytoplasm is amphophilic containing rare azurophilic or purple (primary) granules and many fine lilac or pale orange/pink specific granules.

0.9% of participants incorrectly identified the arrowed cell as a reactive lymphocyte. Reactive lymphocytes show a wide range of morphology but are usually round to ovoid to irregular cells ranging from 10 to 25 µm in size with an N:C ratio that varies from 3:1 to 1:2. Nuclei are round to oval with moderately condensed chromatin (giving it a smeared appearance), and absent or indistinct nucleoli. They contain abundant pale gray-blue cytoplasm. Granules, if present, are usually small and few in number.

Clinical Presentation:

This peripheral blood smear is from a 75-year-old woman with a history of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia presenting with fatigue and noted to have a monoclonal spike on SPEP/SIFE (IgM kappa = 2.81 g/dL). Laboratory data include: WBC = 3.4 x 10E9/L; RBC = 3.20 x 10E12/L; HGB = 9.8 g/dL; HCT = 29.5%; MCV = 92 fL, PLT = 261 x 10E9/L; and RDW = 13%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Lymphoplasmacytic lymphoma with rouleaux formation

Lymphoplasmacytic lymphoma (LPL) is a rare neoplasm of small mature B-lymphocytes, plasmacytoid lymphocytes, and plasma cells usually involving bone marrow and, less commonly, spleen and lymph node. LPL accounts for approximately 1% of hematologic malignancies with an incidence of 8.3 cases per 1 million persons per year. The majority of LPL patients present with a circulating monoclonal IgM that leads to hyperviscosity syndrome and rouleaux formation by red blood cells on peripheral blood smears. Bone marrow involvement by LPL with any concentration of monoclonal IgM is referred to as Waldenstrom macroglobulinemia.

Examples of conditions associated with rouleaux formation

Rouleaux formation refers to a linear arrangement of four or more red blood cells that resemble a stack of coins seen on a peripheral blood smear. When noted in only the thick area of a blood film smear rouleaux formation is a normal finding and not associated with any disease process. True rouleaux formation is present when seen in the thin area of a blood smear and is often associated with a proteinaceous, blue-staining background. True rouleaux formation is a consequence of increased amounts of plasma proteins, primarily fibrinogen, and immunoglobulins, both polyclonal (seen variety of infectious and inflammatory disorders) and monoclonal. Rouleaux formation associated with a monoclonal paraprotein (M-protein) can be seen in monoclonal gammopathy of unknown significance, multiple myeloma, amyloidosis, and lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia.

Question 1. Rouleaux formation refers to the following:

A. Increased erythrocyte sedimentation rate
B. Linear arrangement of four or more plasmacytoid lymphocytes
C. Linear arrangement of four or more red blood cells that resemble stack of coins
D. Presence of monoclonal gammopathy

Typical findings and diagnostic approach in patients with monoclonal paraprotein

An M-protein, or paraprotein, is a monoclonal immunoglobulin secreted by neoplastic cells indicating the presence of an underlying plasma cell or lymphoproliferative disorder. Evaluation for the presence of M-protein is usually performed using electrophoretic techniques on serum or urine (SPEP or UPEP, respectively) supplemented by immunofixation (SIFE/UIFE) to confirm monoclonality and determine the immunoglobulin heavy and light chain class. SPEP should be considered in any patient with unexplained anemia, signs of hyperviscosity, red blood cell agglutination, or other symptoms in whom multiple myeloma, lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia (LPL/WM), primary amyloidosis or related disorders are suspected.

Diagnosis of LPL/WM is most commonly associated with the presence of IgM monoclonal paraprotein, clonal mature B cells, and plasma cells in bone marrow (or, less commonly, in spleen, lymph nodes, or blood) and *MYD88* L265P mutation. The differential diagnosis for LPL/WM includes other small mature B-cell lymphomas with plasmacytic differentiation and multiple myeloma, which can be distinguished by immunophenotyping and genetic findings. In addition, multiple myeloma with an IgM paraprotein is rare, accounting for less than 1% of patients.

Question 2. True or false statement: The presence of serum IgM monoclonal paraprotein is sufficient for a diagnosis of lymphoplasmacytic lymphoma.

A. True
B. False

Natural history of lymphoplasmacytic lymphoma

LPL demonstrates an indolent clinical course with median survival of approximately 10 years. Inferior survival is associated with advanced age, peripheral blood cytopenias, poor performance status, and high serum beta-2 microglobulin and IgM levels. Treatment is indicated in patients with symptomatic disease, which includes symptoms related to the extent of disease involvement (eg, constitutional symptoms, cytopenias), hyperviscosity due to high IgM levels, symptoms due to associated conditions (eg, amyloid, cryoglobulinemia, hemolytic anemia, cold agglutinin), and paraneoplastic neuropathy. The risk of progression to symptomatic disease is greater in the first five years after diagnosis with an estimated annual rate of progression of 4 - 15%.

Question 3. The following findings in a patient with LPL/WM are associated with poor outcome and disease progression:

A. Advanced age, low hemoglobin values, high serum IgM levels
B. Low disease burden, low serum IgM and normal platelet and hemoglobin values
C. Normal serum beta-2 microglobulin levels and low disease burden
D. Young age, low serum IgM levels, and skin rash

Olga Pozdnyakova, MD, PhD
Hematology and Clinical Microscopy Committee

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Answers to Questions:

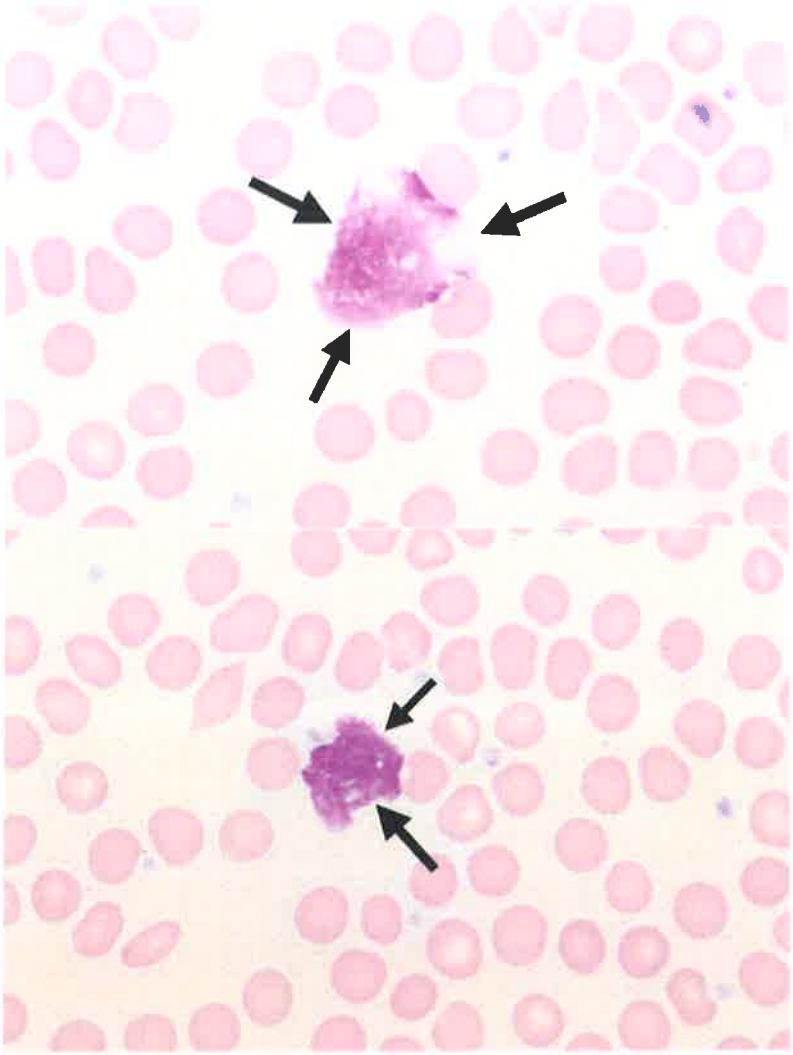
Question 1: C. Linear arrangement of four or more red blood cells that resemble stack of coins
Rouleaux formation is a linear arrangement of four or more red blood cells that resemble stack of coins. True rouleaux formation is present when seen in the thin area of a blood smear and is often associated with a proteinaceous, blue-staining background.

Question 2: B. False
The presence of serum IgM monoclonal paraprotein should prompt an evaluation for lymphoplasmacytic lymphoma, however, by itself is not sufficient for this diagnosis. Diagnosis of lymphoplasmacytic lymphoma is most commonly associated with the presence of IgM monoclonal paraprotein, clonal mature B cells and plasma cells in bone marrow (less commonly, in spleen, lymph nodes or blood), and *MYD88* L265P mutation.

Question 3: A. Advanced age, low hemoglobin values, high serum IgM levels
Inferior outcomes in patients with lymphoplasmacytic lymphoma are associated with advanced patient's age, peripheral blood cytopenias, poor performance status, and high serum beta-2 microglobulin and IgM levels.

Cell Identification

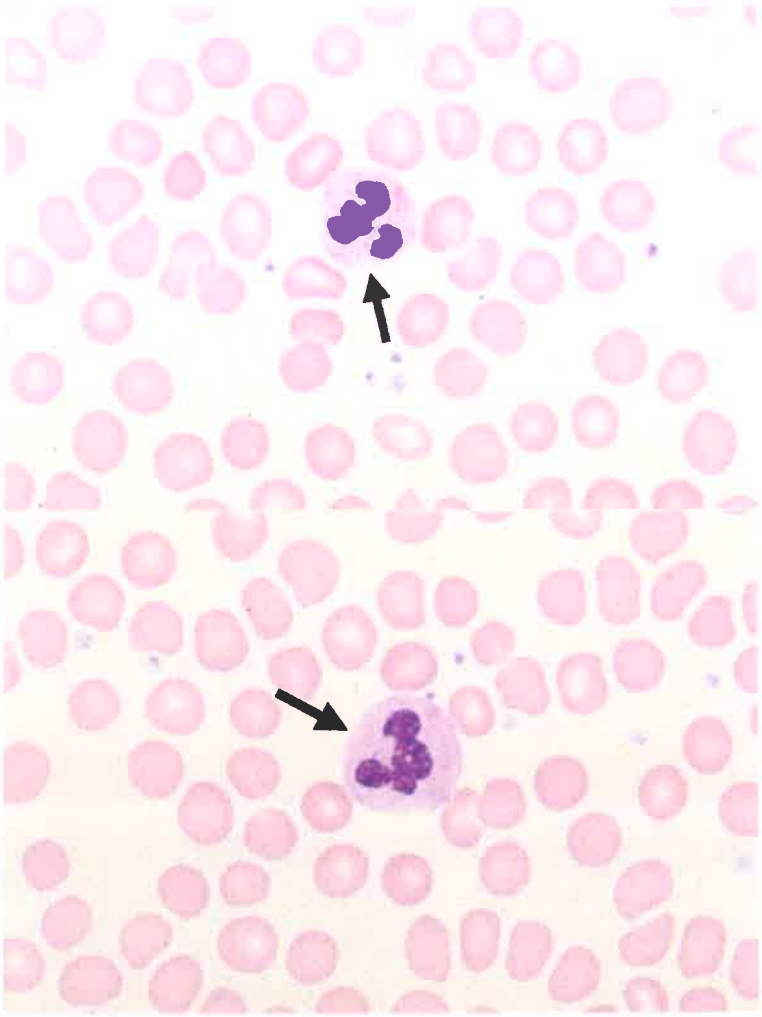
VPBS-14



Identification	Participants No.	Participants %	Evaluation
Basket cell/smudge cell	1155	99.1	Educational
Stain precipitate	8	0.7	Educational
Cryoglobulin	1	0.1	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational

The arrowed cells are basket cells/smudge cells, as correctly identified by 99.1% of the participants. These cells are commonly seen in instances where cells are fragile and easily damaged during the peripheral smear making process. The basket appearance is due to spreading of the chromatin strands of a condensed nuclear remnant. Typically, smudge cells are lymphocytes, but a lack of cytoplasm fails to provide clues to the cell of origin. These cells are most commonly seen in chronic lymphocytic leukemia and infectious mononucleosis, but they can be present in any condition that leads to lymphocyte fragility.

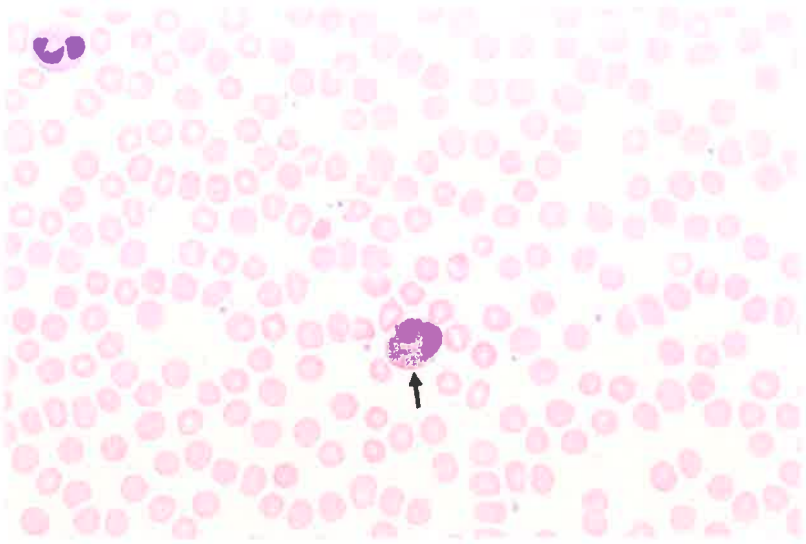
VPBS-15



Identification	Participants No.	Participants %	Evaluation
Neutrophil, segmented or band	1141	97.9	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	10	0.9	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	7	0.6	Educational
Neutrophil with hypersegmented nucleus	6	0.5	Educational
Neutrophil, polyploid	1	0.1	Educational

The arrowed cells are neutrophil, segmented or band, as correctly identified by 97.9% of the participants. Neutrophils are round to oval in shape, 10 - 15 µm in diameter, and have pale pink cytoplasm with specific granules. The nucleus has highly condensed chromatin that is segmented or lobated. Typically, neutrophils have three to five nuclear lobes that are connected by a thin filament that lacks internal chromatin. These thin dark filaments are what separates a segmented neutrophil from a band neutrophil, but for the purposes of proficiency testing, differentiating the two is not required.

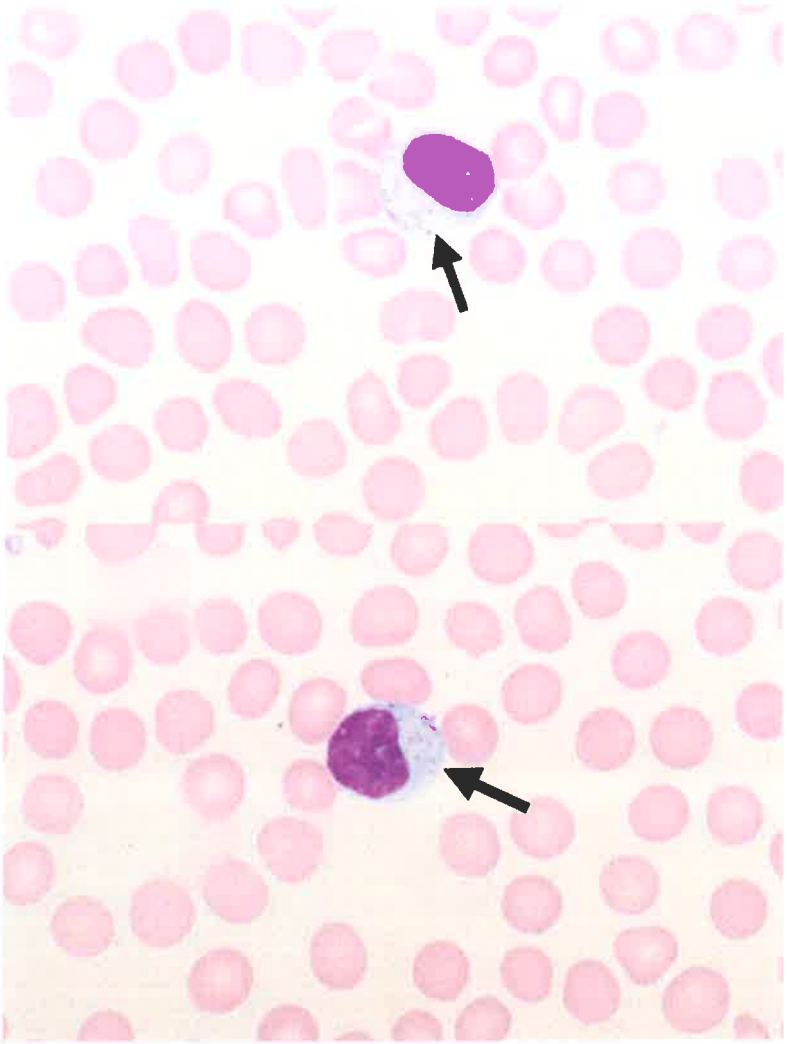
VPBS-16



Identification	Participants No.	%	Evaluation
Eosinophil, any stage	1162	99.7	Educational
Basophil, any stage	1	0.1	Educational
Erythrocyte, normal	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational

The arrowed cell is an eosinophil, as correctly identified by 99.7% of the participants. Eosinophils are round to oval in shape, 10 - 15 µm in diameter, and have cytoplasm that is evenly filled with coarse orange-red granules. The granules are uniform in size, rarely overlying the nucleus, and are refractile by light microscopy. Mature eosinophils have a nucleus with compact chromatin that segments into two or more potato-shaped lobes that are connected by a thin filament. Typically, two lobes are seen, but occasional cells will have three, four, or five lobes.

VPBS-17



Identification	Participants No.	%	Evaluation
Lymphocyte, large granular	932	80.0	Educational
Lymphocyte	200	17.2	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	18	1.6	Educational
Neutrophil, myelocyte	6	0.5	Educational
Immature or abnormal cell, would refer for identification	3	0.3	Educational
Monocyte	2	0.2	Educational
Howell-Jolly body	1	0.1	Educational
Neutrophil, metamyelocyte	1	0.1	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.1	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body	1	0.1	Educational

The arrowed cells are large granular lymphocytes, as correctly identified by 80.0% of the participants. Large granular lymphocytes are medium to large cells compared to a typical lymphocyte which is small and ranges from 7 - 15 µm in diameter. These cells have round nuclei with dense chromatin and abundant clear/lightly basophilic cytoplasm. Within the cytoplasm are small and course azurophilic

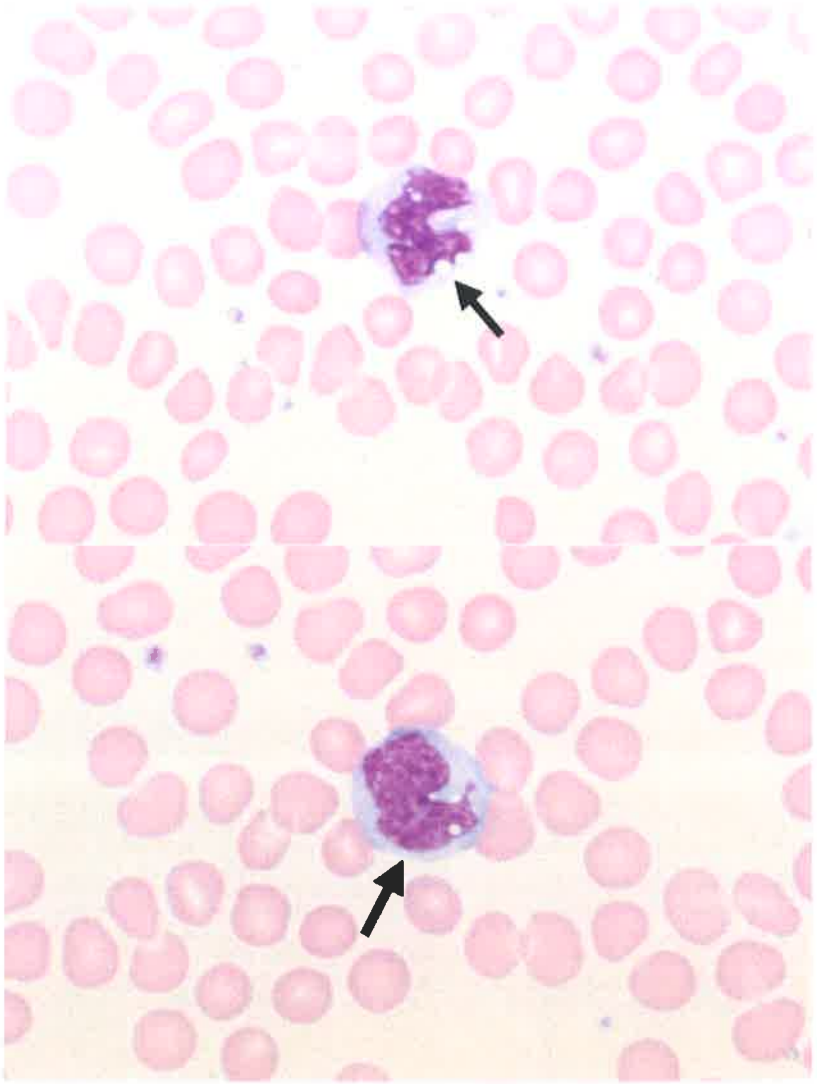
VPBS-17, (cont.)

VPBS-17 Discussion, Cont'd:

granules that are variably distributed. Small numbers of these cells can be seen in normal individuals, but they are often increased in association with reactive lymphocytes which can be seen in infectious processes, drug reactions, and in response to major stressors to the body's immune system. Studies looking at the cell surface markers of large granular lymphocytes show them to be natural killer cells or suppressor/cytotoxic T-lymphocytes.

Approximately 17.2% of participants incorrectly identified the arrowed objects as a lymphocyte and approximately 1.6% of participants incorrectly identified the arrowed object as a lymphocyte, reactive. In contrast to large granular lymphocytes, lymphocytes are generally smaller with a N:C ratio ranging from 5:1 to 2:1. They have a scant amount of pale blue to moderately basophilic cytoplasm, which is agranular. The arrowed cells have variably distributed course azurophilic granules. Reactive lymphocytes can have a wide range of cellular sizes and shapes, as well as nuclear sizes, shapes, and chromatin patterns. The most common type of reactive lymphocyte is a Downey type II cell which has round to oval nuclei, moderately condensed chromatin, indistinct nucleoli, and abundant pale gray-blue cytoplasm. These cells can occasionally have granules, but they are usually small and few in number. Often, these reactive lymphocytes will have an amoeboid cytoplasm that partially surrounds adjacent red cells with a darker-staining furled margin, a feature which is not present in the arrowed cells

VPBS-18



Identification	Participants No.	%	Evaluation
Monocyte	1153	99.0	Educational
Monocyte, immature (promonocyte, monoblast)	6	0.5	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	3	0.3	Educational
Neutrophil, metamyelocyte	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational
Platelet, giant (macrothrombocyte)	1	0.1	Educational

The arrowed cells are monocytes, as correctly identified by 99.0% of the participants. Monocytes are typically round and 12 - 20 µm in diameter. Pseudopod-like cytoplasmic extensions can be seen. These cells have abundant gray-blue cytoplasm that contains vacuoles or fine azurophilic granules. The nucleus has condensed chromatin that is often indented but can be folded or band-like. In general, nucleoli are absent, but occasional forms can contain a small inconspicuous nucleolus.

Clinical Presentation:

This peripheral blood smear is from a 58-year-old woman presenting with shortness of breath and pain in the chest and jaw. Laboratory data includes: WBC = 16.1 x 10E9/L; RBC = 4.50 x 10E12/L; HGB = 14.0 g/dL; HCT = 44.0%; MCV = 98 fL; PLT = 294 x 10E9/L; and RDW = 14%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Mild leukocytosis secondary to myocardial infarction

A myocardial infarction (MI), more commonly known as a "heart attack," results from prolonged severe ischemia leading to death of cardiac muscle, ensuing systemic inflammation, and detection of abnormal cardiac biomarkers on laboratory evaluation.

Acute MIs are medical emergencies that necessitate immediate simultaneous use of multiple therapies to save cardiac tissue. Characteristic patterns seen on an electrocardiogram (ECG) – like "ST segment" elevations, depressions, etc – help to classify the type of acute MI. An "ST-elevation" myocardial infarction (STEMI) indicates complete blood vessel occlusion (mostly through coronary artery plaque rupture), necessitating immediate percutaneous coronary intervention in the cardiac catheterization laboratory. On the other hand, a "non-ST elevation" MI (NSTEMI), can be medically managed with antiplatelet therapy, aspirin, and anticoagulants.

Approximately 1.5 million people in the United States suffer an MI annually. The patient in this clinical vignette experienced an NSTEMI, which manifested in the CBC as a mild leukocytosis, with elevation in all WBC components, leading to mild absolute neutrophilia, lymphocytosis, monocytosis, eosinophilia, and basophilia.

Diagnosis of myocardial infarction

The American Heart Association and the World Heart Federation define acute MI as "the presence of acute myocardial injury detected by abnormal cardiac biomarkers in the setting of evidence of acute myocardial ischemia." According to this definition, there should be a "rise and/or fall of cardiac troponin (cTn) values above the 99th percentile as well as one of the following: symptoms of myocardial ischemia (chest pain, shortness of breath, sweating, nausea, and vomiting), new ischemic changes on electrocardiogram, imaging evidence of myocardial death, or identification of coronary artery thrombus by angiography or autopsy."

Inflammatory response in myocardial infarction

Myocardial necrosis (ie, infarct) induces complement activation and free radical generation leading to a cytokine cascade initiated by tumor necrosis factor-alpha (TNF-α) release. Upon reperfusion of the area of necrotic cardiac muscle cells, a significant inflammatory reaction occurs, leading to a peripheral blood leukocytosis, often including neutrophilia and monocytosis. First, interleukin (IL)-8 and C5a cytokines recruit neutrophils to the area of injury, exerting cytotoxicity and further potentiating the inflammatory response. The infarcted myocardium also upregulates monocyte chemoattractant protein (MCP-1), leading to recruitment of mononuclear cells to the injured areas. Monocyte-derived macrophages and mast cells are essential for effective repair of injured myocardium and scar formation, as they produce cytokines and growth factors that lead to fibroblast proliferation and neovascularization.

Question 1. Why are monocytes essential in the inflammatory response in myocardial infarction?

A. Monocytes are not important for the inflammatory response in myocardial infarction
B. They aid in repairing injured myocardium and help with scar formation
C. They are the first inflammatory cells to arrive after myocardial necrosis
D. They initiate the cytokine cascade

Peripheral blood white blood cell (WBC) findings in myocardial infarction

Leukocytosis is defined as a WBC count greater than 11 x 10E9/L (or above the defined reference interval based on a laboratory's patient population) and can be seen in many conditions including infection, inflammation, stress, drug reaction, and malignancy. In the case of an MI, there is a combination of a systemic inflammatory reaction as well as a stress response leading to increased circulating WBCs, typically including neutrophils, lymphocytes, and/or monocytes. In these instances, neutrophils may sometimes display toxic granulation and Döhle bodies. Also, a mild increase in large granular lymphocytes can be seen in addition to reactive lymphocytes.

Question 2. Which of the following is a common peripheral blood finding in patients experiencing an acute myocardial infarction?

A. Anemia with circulating nucleated red blood cells
B. Circulating blasts
C. Plasma cells
D. Reactive monocytosis

Rebecca Kunak, MD,
Hematology and Clinical Microscopy Committee

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Answers to Questions:

Question 1: B. They aid in repairing injured myocardium and help with scar formation
Monocytes are essential in the inflammatory response after myocardiac infarction. Damaged heart muscles release monocyte chemoattractant protein (MCP-1), which leads to recruitment of monocytes to injured areas. Monocyte derived macrophages and mast cells produce cytokines and growth factors that help to repair the damaged cardiac myocytes. These cytokines and growth factors also lead to increased fibroblasts, which actually form the scar, as well as create new blood vessels (ie, neoangiogenesis) that help to restore blood flow. TNF-α initiates the cytokine cascade and neutrophils are the first inflammatory cells to arrive after myocardial damage due to a myocardial infarction.

Question 2: D. Reactive monocytosis
Reactive monocytosis is associated with multiple conditions including acute and chronic inflammation, acute myocardial infarction, carcinomas, splenectomy, and hypothyroidism. Anemia with circulating nucleated red blood cells can be seen in association with hemolysis, marrow regeneration, bone marrow damage, or stress. Circulating plasma cells and blasts are atypical in peripheral blood and would prompt a differential diagnosis that could include hematologic neoplasia.

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